

Supplementary Materials for

Arctic-Adapted Dogs Emerged at the Pleistocene-Holocene Transition

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Materials and Methods

Modern Greenland dog samples

A total of ten modern biological samples of tissue or DNA extract was obtained from biobanks at the Greenland Institute of Natural Resources, Institute of Bioscience at Aarhus University or Department of Veterinary and Animal Sciences at the University of Copenhagen. Specifically, these ten samples consist of two samples from each of five Greenland cities or villages, specifically, Qaanaaq, Ilulissat, Aasiaat, Sisimiut from West Greenland and Tasiilaq, East Greenland.

The Zhokhov site, background and sample

The site has probably been a base-camp at the centre of a vast mobility network in which dog sledding would have been useful. Several wooden remains resembling dog sled parts have been excavated from the same age context at the Zhokhov site (Fig. S1). The Zhokhov dog specimen (Zh-03-97) ((CGG6)) was excavated at the Zhokhov site, Zhokhov Island, New Siberian Islands in 2003 by Vladimir Pitulko. This specimen of a left mandible (Fig. S2) was directly dated to 8529 ± 30^{14} C years before present (MAMS-24239, calibrated to ca. 9514.5 years before present, using OxCal v4.2.4. (*27*)).

The Yana site, background and sample

The Yana (Y06-NP-18994) ((CGG23)) left mandible specimen (Fig. S3) was excavated from the Yana site by Vladimir Pitulko and Elena Y. Pavlova in 2008-2012, and collected directly from permafrost deposits. This specimen was directly dated to $28,830 \pm 130^{14}$ C years before present (MAMS-24246, calibrated to ca. 33,019.5 years before present, using OxCal v4.2.4. (*27*)).

Methods and analysis

DNA extraction and sequencing

All DNA pre-amplification work on the Zhokhov and Yana samples was performed in ancient DNA facilities at the Centre for GeoGenetics, University of Copenhagen (Denmark) following ancient DNA guidelines (*28*). For ancient DNA extraction, a piece of a tooth from each specimen was crushed and then extracted in a urea-proteinase K buffer following (*29*). DNA in the digest was bound to a MinElute column (Qiagen, Hilden, Germany) in combination with a binding apparatus as described in (*30*) using a binding buffer following (*31*) in a sample:buffer ratio of 1:10. The column was subsequently treated according to the manufacturer's guidelines. Modern DNA extraction was performed using the Qiagen Blood and tissue kit (Qiagen) according to the manufacturer's guidelines. Modern DNA samples were fragmented prior to library preparation using a Bioruptor NGS device (Diagenode, Liège, Belgium). All extracted DNA was converted into double-stranded sequencing libraries using the NEBNext DNA Sample Prep Master Mix Set 2 (E6070 - New England Biolabs Inc., Beverly, MA, USA) and the "single-tube" library building protocol BEST as described in (*32*). Libraries were sequenced using Illumina HiSeq 2000 and 2500 (Illumina, San Diego, CA, USA) platforms at the National High-throughput Sequencing Center, Copenhagen, Denmark and BGI-Europe, Copenhagen, Denmark.

Quality control and alignment

We used the PALEOMIX (*33*) pipeline to process short reads obtained for all ancient and modern samples, including the samples that were obtained from previously published studies. As the first step in the pipeline, we trimmed the reads and removed adapters using

AdapterRemoval2 (*34*). Paired-end reads overlapping more than 10 base pairs - calculated using the sequences at the 3' end of the first read and the 5' end of the second read of the pair - were merged into a single long read (--collapse option). Reads shorter than 25 bp (after removing adapter sequences) were discarded. These processed reads were mapped against the wolf reference genome (*35*) and to the dog reference genome (CanFam3.1) using the alignment tool, bwa aln (v0.7.15; aln algorithm) (*36*), or as in Leathlobhair *et al*. (*3*) (specifically for the CTVT work). Duplicate reads and reads that mapped to multiple locations in the reference genome were discarded using the Picard set of tools (v1.128, https://broadinstitute.github.io/picard). In order to improve the local mapping of reads that span indels, we used GATK (v3.8.0) (*37*, *38*) to perform an indel realignment step on the mapped reads for each of the samples. Since we do not have a set of curated indels in the species of interest for these samples, we performed the indel realignment step using no external indel database. All analyses were performed using the alignments against the wolf reference genome (*35*), unless stated otherwise. The wolf reference genome was used in order to avoid potential reference biases when comparing the ancient Zhokhov sample to present-day dog genomes (*5*).

Assessing DNA damage patterns

We used mapDamage (v2.0.6) (39) to assess the type specific error rates estimated and aDNA damage patterns in the two ancient samples sequenced in this study. Mapped reads from the Zhokhov and Yana samples showed an increased proportion of C to T and G to A substitutions at the 5' and 3' read ends, respectively (Fig. S4). Additionally, mapDamage (v2.0.6) (*39*) was used to rescale the quality scores of bases inferred to be affected by DNA damage in the Zhokhov and Yana samples, as well as ancient reference samples, viz., the ~34,900 years BP Siberian Taimyr wolf (*5*), the ~4800 years BP Irish Newgrange dog (*40*), the ~4700 years BP German Cherry Tree Cave dog (41) , the ~7000 years BP German Herxheim dog (41) , the ~4200 years BP Newfoundland Port au Choix dog (*3*), the ~1100 years BP Alaskan Uyak dog (*3*), the ~960 years BP Virginian Weyanoke Old Town 1 dog (*3*) and the ~1100 years BP Virginian Weyanoke Old Town 2 dog (*3*).

Error rates

To further assess the quality of the aDNA data, we estimated type specific error rates using ANGSD (v0.921) as described in Orlando, *et al.* (*28*) for the Zhokhov and Yana samples. Majority count consensus sequences for the Andean fox and Greenland wolf genomes were used as the ancestral and perfect genome, respectively. In each case, the consensus sequence was created using ANGSD at sites with a minimum depth of coverage of 3, reads with minimum mapping quality of 30 and bases with minimum base quality of 20. Figure S5 shows the error rates estimated for Zhokhov and Yana samples as well as for reference ancient samples (Newgrange, Cherry Tree Cave dog, Herxheim dog, 3 American pre-contact dogs and Taimyr wolf) and five modern samples used in this study, shown as comparison. Overall, Zhokhov and Yana genomes display error rates comparable to those of other sequenced ancient dog and wolf genomes (Fig. S5). These errors are mostly due to increased C to T and G to A substitutions caused by aDNA damage. To account for these errors specific to ancient samples, we restricted the analysis described below to transversions, except when using called genotypes.

Genotype calling

We performed genotype calling on each of the ancient and modern samples included in the study independently, using the HaplotypeCaller algorithm implemented in GATK v3.8 (*37*). We called genotypes for each sample filtering out bases with base quality less than 20 and reads with mapping quality less than 30. This minimally filtered set of genotypes was used for Population Branch Statistics (PBS) and pairwise F_{st} analyses, with further filtering being performed in each analysis.

Reference datasets used

We used a combination of previously published whole genome sequences and genome wide SNP chip data as reference material. References and details for whole genome data are given in Table S1 and for genome wide SNP chip data is given in Table S3.

Principal Component Analysis

We performed a principal component analysis (PCA) using smartpca (*42*, *43*), from the eigensoft suite of tools (v7.2.1, https://github.com/DReichLab/EIG), to explore the affinity between Zhokhov and Yana genomes to a dataset of ancient and present-day dogs, present-day wolves and the Taimyr wolf (Table S1). For each sample and each site sampled one allele by creating a majority count consensus sequence. This consensus was created using ANGSD (v0.921) in sites with a minimum depth of coverage of 3, reads with a minimum mapping quality of 30 and sites with a minimum quality of 20. This approach allowed us to incorporate samples with heterogeneous depths of coverage. Sites with a missingness greater than 20% and minor allele frequency $(MAF) < 0.05$ were excluded. Additionally, to reduce the bias introduced by aDNA damage, we only included transversion in the analysis. The final dataset consisted of 2,200,623 sites. Smartpca was run using the *lsqproject* option to be able to include low coverage, high missingness samples in this analysis.

Pairwise distances

Pairwise distances between the Zhokhov genome and the dog samples in the whole-genome panel (Table S1) were calculated as the fraction differences between pairs of samples using PLINK 1.9 (*44*) and a random allele for each individual. A similar sampling and filtering approach and dataset as the one described for the PCA were used.

Admixture analyses using whole genome data (and Genotype likelihoods)

Identifying variant sites and calling genotype at these sites has been shown to introduce biases when the sequencing data used for such analyses is very heterogeneous (*45*). In order to avoid these issues, we performed a subset of the analyses using randomly sampled bases in some cases, and genotype likelihoods in others. The genotype likelihoods at variant sites were computed in ANGSD (v0.921) (*46*) using the aligned reads obtained from PALEOMIX, under the model proposed in *samtools* (v1.2) (*36*). Nucleotides with base qualities lower than 20 and reads with a mapping quality lower than 20 were discarded. Sites with data at fewer than 95 out of the 99 samples were excluded. Finally, only sites with an estimated MAF greater than 0.05 were retained. We used NGSAdmix (*47*) on the genotype likelihoods computed in ANGSD to estimate the ancestry clusters, and the admixture proportions (Fig. S_6) in our dataset using ~8.5 million SNPs. We explored the structure in our samples by running the admixture analyses with a different number of estimated ancestry clusters, ranging from 2 to 10 clusters. To ensure

convergence to the global maximum, the analysis was repeated 200 times, and the replicate with the best likelihood was chosen.

Admixture analysis using SNPs data

We compared the Zhokhov genome to a genotype diversity panel comprising multiple dog breeds using a model-based clustering algorithm. To allow for the inclusion of more sled dogs and reference individuals to complement full genomes, Illumina CanineHD 185,805 array genotyped data from 192 dogs was included solely in Figure S8 (Table S3). The Zhokhov genome was incorporated into the panel as diploid called genotypes. From each of the dog breeds included we randomly selected 10 individuals when more than 10 were available in the dataset, except for the sled dogs, for which we included all individuals available (Table S3). We kept sites with MAF≤0.01 and maximum missingness of 10%, and the final dataset consisted of 214 dog samples and 130,253 sites. ADMIXTURE was run on the final dataset assuming 2 to 12 clusters ($K=2-12$), and for each K value, we ran 50 replicates starting from different seeds (Fig. S7A). Additionally, we obtained cross-validation errors for the best replicate of each K (Fig. S7B).

Admixture graphs using TreeMix

We used TreeMix (*48*) to explore the broad phylogenetic context of the Zhokhov sample with respect to ancient and present-day wolf and dog genomes. From the whole-genome dataset, we selected samples representing the main ancestry groups (Table S1 - Selected): Eurasian grey wolves (n=19), Arctic grey wolves (n=6), Mexican grey wolves (n=2), Alaskan grey wolves $(n=2)$, Pleistocene wolves $(n=2)$, Asian dog breeds $(n=6)$, European dog breeds $(n=5)$, Siberian and Alaskan huskies (n=5), Alaskan Malamute dogs (n=1), Greenland village dogs (n=10), American pre-contact dogs $(n=1)$, the Zhokhov dog, and Coyote $(n=2)$, which was used as outgroup. For each sample and site, we randomly sampled one allele by building a majority count consensus sequence using ANGSD with a similar filtering approach as the one described in the MDS section. Allele frequencies were estimated on the different clusters as described in Table S1 (Selected). Additionally, we excluded invariant sites, transitions and sites with missing data. TreeMix was run on the final dataset which consisted of 766,082 sites assuming 0 to 7 migration edges (m=0-7). For each migration, we ran 100 replicates starting at different seed values and chose that with the highest likelihood for each value of *m* (Fig. S8).

CTVT

We analysed data from two publically available CTVT genomes (and their respective host genomes) (*49*), which possess a low level of host contamination and which were previously genotyped in Leathlobhair *et al*. (*3*). We used the same set of variable sites (~2.03M SNPs, including ~600K transversions) described in Leathlobhair *et al*. (*3*) for all following analyses including CTVT genomes. Briefly, this set was selected in order to minimize the effect of somatic mutations, host contamination, and to retain only regions of the dog reference genome that are diploid in cancer cells, as previously described in (*49*). We used plink v1.9 (*50*) to compute an Identity By State (IBS) matrix using all 2.03M SNPs. This matrix was used to build a neighbour joining tree (NJ) using the R package "ape" (*51*). The resulting tree shows that the CTVT genomes (C_79T and C_24T) cluster with the Port au Choix dog in clade basal to the Zhokhov and sled dogs (Fig. \overline{S} 9). We then computed outgroup f3-statistics as f3(CTVT, X; Andean Fox) using ADMIXTOOLS (*52*) where X is any other dog population to quantify the

amount of genetic drift shared between the CTVT other dogs using only transversions (Fig. S10). This analysis recapitulated the same pattern, indicating that the CTVT genomes are indeed closer to the Port au Choix genome. Reassuringly, the CTVT genomes did not cluster with their respective hosts (H_79T and H_24T in Fig. S10).

D-statistics

D-statistics as implemented in ADMIXTOOLS (*52*) were used to evaluate the shared ancestry and gene flow between the Zhokhov genome, modern and ancient dog and wolf genomes (Figs. S11 - S15). In order to incorporate genomes with heterogeneous depth of coverage, we randomly sampled alleles from a majority count consensus sequence at every position and for each sample. Reads with quality lower than 30, bases with quality lower than 20 and sites with coverage lower than 3 were discarded from the analysis. Additionally, transitions were removed from the final dataset to avoid incorporating errors derived from ancient DNA damage in the Zhokhov genome and other ancient samples used. A weighted block jackknife procedure over 1Mb blocks was used to assess the significance of the tests. Deviations from D=0 with a Z-score above or below 3.3 ($|Z| > 3.3$) were presumed significant. For most D-statistics tests, we use the Andean fox (*Lycalopex culpaeus*) genome as an outgroup to avoid conflicting results caused by the frequent interbreeding between canids (*53*).

a) Zhokhov is more closely related to dogs than to wolves

To test if the Zhokhov genome form a clade with dogs to the exclusion of wolves, we computed a D-statistic of the form D(Zhokhov, H2; Croatian wolf, Andean fox), where H2 corresponds to dog genomes in the reference panel. In each case, we find support for the Zhokhov genome forming a clade with dogs to the exclusion of the Croatian wolf (Fig. S11). Conversely, we were able to reject the alternative hypothesis that the Zhokhov genome form a clade with the Croatian wolf or falls basal to wolves and dogs $(|Z|>40)$.

b) Zhokhov dog falls basal to the Greenland sled dogs and other sled dogs

Admixture graphs obtained from TreeMix suggested Zhokhov dog lineage diverged from the ancestor of present-day Greenland sled dogs and other sled dogs (Alaskan malamute, Alaskan husky and Siberian husky). To further confirm its phylogenetic positioning within dogs, we computed a D-statistic of the form D(H1, H2; Zhokhov, Andean fox), where H1 and H2 represent all possible pairs of Greenland sled dogs and other sled dogs (Fig. S12). We found the Zhokhov dog to be symmetrically related to every pair of Greenland led dogs and pairs of other sled dogs (|Z| 3.3) however, when comparing Greenland sled dogs with other sled dogs, the ancient Zhokhov sample was found to be significantly closer to Greenland sled dogs ($|Z| > 3.3$). Additionally testing of affinity of modern sled dogs to the Zhokhov dog, using D(Alaskan Husky 1, H2; Zhokhov, Andean fox), further supports more allele sharing between the Zhokhov dog and Greenland sled dogs, than to other sled dogs. Finally, using test D(Greenland sled dog, Siberian Husky 2; H3, Andean Fox), of all ancient dogs in the data, the Zhokhov dog shared most alleles with Greenland sled dogs.

c) Affiliation of Greenland sled dogs and other sled dogs, outside Zhokhov

TreeMix results suggested other sled dogs, particularly Siberian and Alaskan huskies, carry gene flow from other, possibly European, dog breeds (Fig. S8). We used D-statistics to test if this gene flow could explain the significant results obtained in the test. First, we computed a D-statistic of the form D(other sled dog, Greenland sled dog Aasiaat 1; H3, Andean fox), where H3 represent all dogs in the reference panel and Aasiaat 1 was used as a representative of the Greenland sled dogs, to identify the best candidate for the gene flow observed in other (non-Greenland) sled dogs (Fig. S13A). Next we used the form D(sled dog 1, sled dog 2; H3, Andean fox), where H3 represents 4 diverse dogs in the reference panel and sled dog 1 and sled dog 2 represent all possible pairs of sled dogs to identify the best candidate for the gene flow observed in other (non-Greenland) sled dogs (Fig. S13B). The statistics reflect the TreeMix results, finding other sled dogs, particularly Siberian and Alaskan huskies, carry gene flow from other dogs.

d) Affinities between the Pleistocene wolf, the Zhokhov dog and sled dogs

Previous studies have identified allele sharing between a Pleistocene Siberian wolf genome and dogs of especially Arctic context such as sled dogs (*5*), but also in extinct dogs from the Americas before European contact (*3*). We estimated a D-statistic of the form D(sled dogs, Boxer; Taymir/Yana, Andean fox) xy-plot (Fig. 1D and S14), and replicated these findings. However the signal was only significant when using the Yana wolf, likely due to fewer sites when using the lower coverage Taymir wolf genome. Similar to the previously documented Siberian Pleistocene wolf gene flow, we find that the Zhokhov dog shares significantly more alleles with Yana and Taimyr wolf, compared to the boxer dog.

e) Affinities between sled dogs and modern wolves

It is often heard that sled dogs are admixed with wolves and historical observations document such events in Arctic North America and Greenland (*54*, *55*). We tested this notion specifically using Greenland- and other sled dogs against a large panel of modern wolves, including specimens from the entire North American Arctic and Greenland (*53*, *56*) (Fig. S15). To estimate whether Greenland dogs admixed with local wolves since they diverged from their common ancestor with the Eurasian wolves, we computed a D-statistic of the form D(Greenland sled dogs, Portuguese wolf; Arctic wolves, Andean fox), for all Greenland dogs and all Arctic wolves. We found no significant admixture between any of the Greenland dogs and any wolves.

Population size estimates in Greenland sled dogs

We estimated the effective population size of the Greenlandic sled dogs, using the diffusion approximation model for site frequency spectrum (SFS) implemented in the package *moments*. First, we estimated the site frequency spectrum for the Greenlandic sled dogs, using the 11 samples across Greenland. We estimated the 1-D SFS for these sled dogs using *realSFS*, a utility implemented in *ANGSD* v0.921, which employs genotype likelihoods to incorporate uncertainty introduced by low and different coverage sequencing among the 11 samples. Using this SFS, we estimated parameters of a simple demographic model that allows for two population size changes (Fig. S16). The first one at 89,825 years ago, outside the relevant time frame of this study, the second 864 years ago corresponding with the Inuit/Thule culture's introduction of this dog lineage into Greenland. Demographic parameters were estimated under a maximum likelihood model for the SFS under the given class of demographic models.

Heterozygosity

Heterozygosity is often used as a proxy measure for effective population size. Here, we computed heterozygosity for the different whole-genome sequenced samples included in the study, using ANGSD v0.921 and realSFS (a utility tool from ANGSD). To compute heterozygosity, we estimated the single sample site frequency spectrum (SFS) for each sample. Folded SFS was estimated using genotype likelihoods computed using ANGSD. Heterozygosity was computed as the proportion of sites with a minor allele count of 1, which is effectively heterozygosity since the SFS is computed for a single sample. Finally, we obtained bootstrap variance of heterozygosity by obtaining 100 bootstrap estimates of the SFS. Estimated heterozygosity for the different samples (Fig S17A) and populations (Fig. S17B) included in this study showed lowest heterozygosity for Greenland sled dogs, among all non-breed dogs. The excess heterozygosity of other sled dogs, compared to the Greenland sled dogs, can be explained by genetic contributions from European dogs. The low heterozygosity of the Greenland sled dogs is consistent with the population size reduction shown in the demographic inferences (see above section).

Genetic adaptations in sled dogs Population branch statistic

To detect signals of positive selection, we used the population branch statistic (PBS) (*10*), which identifies alleles that have experienced strong changes in frequency in one population (sled dogs) relative to two reference populations (a sister population, such as other dogs [non-sled dogs], and an outgroup, such as wolves). Thus, PBS identifies genomic regions highly differentiated in the sled dog branch - a signal that can be suggestive of positive selection. PBS has previously proven useful for identifying population specific selection in humans $(10, 25)$. We computed F_{ST} between the following three populations: sled dogs, other dogs and wolves (Table S1 - Selected). F_{ST} was computed using the Hudson estimator (57) in windows of 100 kilo-base pairs with a 20 kilo-base pairs slide (Fig 3A) and 25 kilo-base pairs with a 5 kilo-base pair slide (Fig S19). Since we were interested in discovering putative targets of positive selection, we used the alignments to the dog reference genome (See Quality control and alignment), taking advantage of its longer continuity and better gene annotation than the wolf assembly. The three F_{ST} measures were then combined in all approaches to obtain the PBS as described in (*10*), taking as the focal population sled dogs (Fig 3A) and other dogs (Fig S18). Genes overlapping with extreme outlier regions are listed in Table S4-S5.

XP-CLR

XP-CLR (*58*) was computed across the autosomes using xpclr 1.0 (https://reich.hms.harvard.edu/software) with parameters "*-w1 0.0075 200 500 chromosome -p0 0.95*". The two populations compared were sled dogs $(n=17)$ and other dogs $(n=61)$ (Table S1), as defined in our PBS scan. XP-CLR around the autosomal genomic regions above the 99.95th percentile of PBS can be found in Figure S23.

Functional enrichment in high PBS regions

We computed enrichment of gene ontology (GO) terms in the regions of the genome that displayed high PBS values when using the Greenland sled dogs as the focal population, to identify biological processes or molecular functions that genes in this region represent. To compute enrichment of GO terms in this region, we used the interval enrichment program, inrich (*59*), with dog specific annotations from the GO database amigo (http://amigo.geneontology.org/) and gene annotations from ensembl (. Further, we computed the enrichment using two different PBS thresholds - 0.451 and 0.328 (corresponding to the 99.95th and 99.5th percentile of the empirical distribution) - that resulted in 14 and 113 non-overlapping intervals respectively. The enriched GO terms with empirical p-value < 0.05 are shown in Table S6.

Supplementary Figures

Fig. S1. The Zhokhov site sledge remains. (A-I) – sled runner fragments; (**D, E, F**) – upright (**F**) is combined with a sled runner (**G**) in (**H**) – hole with a piece of rope made of animal hair, as an example of using these parts together (**I**); Parts indicated a large sled, making it unlikely that it was pulled by humans, but rather by dogs.

Fig. S2. The Zhokhov dog. Zhokhov dog (Zh-03-97 or CGG6) mandible and size in cm.

Fig. S3. The Yana wolf. Yana wolf (Y06-NP-18994 or CGG23) mandible and size in cm.

Fig. S4. Authenticity of ancient DNA (aDNA) sequencing data. Panels (**A**) and (**B**) show the different substitution rates at the 5' and 3' ends of reads in the Zhokhov dog and the Yana wolf sample respectively. In each panel, the plot on the left shows the substitution rates at the 5' end of reads, whereas the plot on the right shows the substitution rates at the 3' end of reads. The red line shows the C-T substitution rate, which is the most abundant substitution at the 5' end of reads due to ancient DNA damage. Similarly, the blue line shows the G-A substitution, which is the most abundant substitution at the 3' end of reads in both samples.

Fig. S5. Authenticity of ancient DNA (aDNA) sequencing data. Type specific error rates. Type specific error rates estimated using ANGSD for the ancient samples used in this study. Grey bars correspond to the error rates estimated in four modern dog genomes processed using the same pipeline as the ancient data and shown as comparison. Orange bars correspond to reference ancient samples used in this study, red and dark red correspond to the error rates estimated for the Zhokhov and Yana genomes sequenced in this study.

Fig. S6. Extended admixture results from whole-genome data. NGSadmix clustering results obtained for a panel comprising whole-genome data. Individual colors illustrate the inferred admixture components, Ks values indicated at the top. The sample name is given at the left and the relevant clusters are detailed at the right side of the figure.

Fig. S7. Extended admixture results from called genotypes of genomes and genome wide SNP data. (**A**) Clustering results obtained using ADMIXTURE and a genotype panel comprising world-wide dog breeds. The Zhokhov sample was incorporated into the panel as called genotypes. Transitions, sites with >20% missing data and MAF<0.05 were excluded from the analysis leaving a total of 130,253 sites. ADMIXTURE was run assuming 2 to 9 clusters/populations (*K*=2-9). For each *K*, individual bars represent different samples and the colors represent the proportions of each of the inferred components. (**B**) Cross-validation errors obtained for each value of K.

Fig. S8. Phylogenetic placement of Zhokhov sample using TreeMix admixture graphs. TreeMix relationship of major clusters of diversity in the dataset. Admixture graphs computed using TreeMix on a dataset consisting of 66 individuals merged into 15 groups, representing the major groups of wolves (American, Eurasian and Pleistocene wolves) and dogs, and enriched for sled dogs dogs. For each individual a random allele was chosen at each site; transitions, nonpolymorphic sites and sites with missing data were excluded from the analysis (final dataset of 766,082 sites). We fitted from 0 to 7 migration edges. In the right of each subpanel; inferred tree

with admixture edges represented as arrow and colors indicating the fraction of admixture. In the left of each subpanel, a heatmap indicating the residuals obtained from the fitted graph with colors indicating the standard errors of each node.

Fig. S9. CTVT NJ tree. A neighbour joining (NJ) tree illustrating cladistic relationship between CTVT genomes, wolves and dogs. The NJ tree is based on Identity By State and bootstrap values supporting phylogenetic clusters. The tree place the CTVT genomes closer to the Port au Choix dog than to Zhokhov and sled dogs. Further the tumor hosts are correctly placed near European dogs.

Fig. S10. CTVT f3-statistics. f3-statistics testing the relationship between CTVT genomes, wolves and dogs. Shared genetic drift measured by f_3 (Outgroup; Y, X) where X is either Tumor C_79T or C_24T and X is a selection of references (same individuals as in Fig. S10) shown in panel to the right.

between the Zhokhov genome, dogs and wolves. (A) Test showing the Zhokhov genome forms a clade with all dogs to the exclusion of the Croatian wolf (-3.33<*Z*<3.33). On the contrary, tests where (B) the Zhokhov genome is an outgroup to pairs of dogs and the Croatian wolf, or (C) the Zhokhov dog forms a clade with the Croatian wolf to the exclusion of all dogs were all rejected (Z>3.33). D-statistics were computed using whole-genome data and a random allele for each sample as described in the methods section. Transitions were not included in the analysis. Individual points represent the *D* value obtained from each test. Horizontal bars show 1 (first vertical mark) and ~3.3 standard errors. Tree topologies at the top of each panel indicate the null (D=0) and alternative hypotheses (D >0 and D \leq 0) tested.

Fig. S12. D-statistics showing Zhokhov genome falls basal to sled dogs. D-statistics supporting the position of Zhokhov dog as an outgroup to sled dogs. We computed D-statistic tests of the form D(H1, H2; Zhokhov dog, Andean fox), where H1 and H2 represent all possible combinations of sled dog genomes available. Values of D are indicated as individual points. Horizontal bars represent 1 (first mark) or \sim 3.3 standard errors. Tests involving pairs of Greenland sled dogs yielded values of D consistent with the Zhokhov dog being symmetrically related. Most tests involving pairs of Other (non-Greenland) sled dogs resulted in values of D that suggested that either a) the Other (non-Greenland) sled dogs carried admixture from some sample outside the sled dogs or b) the tree topology suggested in the test is incorrect. In Fig. S13, we show that the a) is the most likely explanation. Tests that yielded significant scores (|*Z*|>3.33) are shown in red.

A

Fig. S13. Affiliation of Greenland sled dog and other sled dogs, outside Zhokhov. Dstatistics testing for gene flow from non-sled dog breeds into the sled dogs. **A.** We computed a D-statistic of the form D(Other sled dogs, Greenland sled dog Aasiaat 1; Dogs, Andean fox) in order to test whether Other (non-Greenland) sled dogs carry ancestry from other dogs breeds when compared to Greenland sled dogs. D-statistics were estimated using whole-genome data and a random allele for each sample as described in the methods section. Transitions were not included. Individual points represent the *D* value obtained from each test. Horizontal bars show 1 (first vertical mark) and \sim 3.3 standard errors. Tests indicating significant gene flow between samples in the ingroup and H3 (*|Z|*>3.33) are shown in red. **B.** D-statistic tests showing that sled

dogs resulting in significant deviations from the test D(sled dog, sled dog; Zhokhov dog, Andean fox) (Figure 2A), also resulted in significant gene flow from other dogs. Individual density distributions correspond to the z-scores obtained from all tests of the form D(H1 (left), all possible SDs; admixing dog (top), Andean fox), where the admixing dog is represented by four dogs that yielded significant scores ($|Z| \geq 3.3$) for the test in Figure S13. Points represent the Zscores obtained from each test. Colors indicate whether the dog in H2 is a Greenlandic (orange) or another sled dog (yellow). Dotted lines show the significance threshold $|Z|\geq 3.3$. In brief, tests involving Greenland sled dogs do not show significant gene flow from any of the admixing dogs. In contrast, tests involving other sled dogs yielded significant deviations from D=0 ($|Z|\geq 3.3$).

Fig. S14. Pleistocene wolves D-statistics. D-statistics showing allele sharing between the ancient Taimyr and Yana wolves and sled dogs. D-statistics showing there is excess allele sharing between both Pleistocene wolves (Yana and) most sled dogs, including the Zhokhov and American pre-contact dogs, when compared to the modern grey wolf. Note that, even though not all results yield significant Z-scores (|*Z*|>3.33) when using Taimyr, potentially due to the limited amount of sites resulting from the low coverage data, both Taimyr and Yana show the same pattern. D-statistics were estimated using whole-genome data and a random allele for each sample (see methods section). Transitions were excluded. Individual points represent the *D* value obtained from each test. Horizontal bars show 1 (first vertical mark) and ~3.3 standard errors.

Fig. S15. Modern wolves D-statistics. D-statistics testing for gene-flow between Greenland sled dogs and a wolf from: Greenland, Victoria Island, Baffin Island, Alaska and Siberia. Individual samples are given on the left (H1) or in the top (H3) of each panel. D-statistics were estimated using whole-genome data and a random allele for each sample (see methods section). Transitions sites were excluded. Points indicate the *D* value obtained from the test. Horizontal bars show 1 (longer line) and \sim 3.3 (shorter line) standard errors. We did not find significant gene flow between any of the Greenland sled dogs and Arctic wolves tested.

Fig. S16. Effective population size (Ne) of Greenland sled dogs through time. The demography of the Greenland sled dogs estimated using the diffusion approximation, as implemented in the software package *moments*. **A.** the demographic history of the Greenland sled dogs, with population size indicated by the width of the bar, and time on the y-axis, in years. **B**. The estimated (blue line) and observed (red line) folded site frequency spectra (SFS). **C.** Difference between the observed and estimated SFS. The y-axis shows the deviation of the estimated SFS from the observed SFS (in number of sites).

A

Fig. S17. Heterozygosity estimates. Heterozygosity estimated under a genotype likelihood framework. (**A**) Heterozygosity for each sample is represented by a single bar, and the samples are grouped by population labels. Error bars are not displayed. (**B**) Population heterozygosities plotted using box plots. The median heterozygosity is marked by the horizontal line, while the box marks the first and third quartiles. The whiskers extend from the end of the box 1.5 times the interquartile range, or to the end of the range, whichever is closer. Finally, observations beyond the whiskers are considered outliers. Overall, Greenland sled dogs have the among the lowest diversity in dogs, with the exception of a few other dogs - a Dingo, a Basenji and a Siberian Husky show lower heterozygosity.

Fig. S18. Manhattan plot of PBS with other dogs as the focal population. PBS values in windows of 100 kilo-base pairs using a 20 kilo-base pairs slide. The 99.95th percentile of the empirical distribution is shown as a red dashed horizontal line. Names of genes with highest overlap associated with the peaks are shown. We note other genes not displayed in the figure overlap such regions: chr6: RNPC3, AMY2B, chr16: MGAM, TAS2R38, CLEC5A, COR9A7 and chrX: RPS6KA3, MAP7D2, EIF1AX. The full list can also be found in Table S5.

Fig. S19. Haplotype plots and PBS at outlier regions. Haplotype plots: each row represents an individual, and each column a polymorphic position in the dog genome. Cells are colored by the individual's genotype, dark gray indicates homozygous for the alternative allele, light gray indicates heterozygous positions and white indicates homozygous for the reference allele. PBS scatter plots: values in windows of 25 kilo-base pairs using a 5 kilo-base pairs slide. The percentile of the empirical distribution is shown as a red dashed horizontal line. Names of genes associated with the highest peaks are shown.

Fig. S20. MGAM and AMY2B. Above is the haplotype structure in MGAM. Each row represents an individual, and each column a polymorphic position in the dog genome. Cells are colored by the individual's genotype, dark gray indicates homozygous for the alternative allele, light gray indicates heterozygous positions and white indicates homozygous for the reference allele. The row width for ancient individuals has been increased (Zhokhov, Herxheim, Cherry Tree Cave, Newgrange and Yana). Below is the read depth based estimate of the AMY2B copy number.

Fig. S21. XP-CLR for outlier regions in the autosomes. XP-CLR (blue) around outlier autosomal regions detected in the PBS scan (orange). Dashed lines represent the 99.9th percentile of both measures.

Supplementary Tables

Table S2. D-statistics in figure 1D. All the D-statistics of the form D(H1, Boxer; H3, Andean Fox), computed with all dogs other than Boxer in H1, and with H3 as either the Taimyr wolf or the Yana wolf, are shown below.

H1	H3	Dstat	SE	Z score	Number of sites
Basenji	Taimyr wolf	-0.0232	0.0099	-2.33	11607
India 2	Taimyr wolf	-0.0196	0.0100	-1.96	11763
Uyo	Taimyr wolf	-0.0189	0.0095	-2.00	12841
Ibadan	Taimyr wolf	-0.0158	0.0094	-1.69	12758
India 4	Taimyr wolf	-0.0143	0.0094	-1.52	12519
Ondo	Taimyr wolf	-0.0127	0.0091	-1.39	13289
Egypt 2	Taimyr wolf	-0.0121	0.0100	-1.21	11663
India 1	Taimyr wolf	-0.0096	0.0094	-1.02	12831
Tibetan Mastiff	Taimyr wolf	-0.0080	0.0094	-0.86	13192
Samoyed	Taimyr wolf	-0.0071	0.0102	-0.70	11733
Lapponian Herder	Taimyr wolf	-0.0066	0.0099	-0.66	11701
Afghan	Taimyr wolf	-0.0057	0.0095	-0.60	12711
Jämthund	Taimyr wolf	-0.0055	0.0097	-0.57	11920
Siberian Husky 3	Taimyr wolf	-0.0054	0.0091	-0.59	13593
Peruvian naked	Taimyr wolf	-0.0050	0.0099	-0.50	11483
Papua New Guinea 3	Taimyr wolf	-0.0047	0.0104	-0.45	10302
Lebanon 3	Taimyr wolf	-0.0045	0.0105	-0.43	10266
Lebanon 1	Taimyr wolf	-0.0040	0.0109	-0.37	9243
Jalingo City	Taimyr wolf	-0.0040	0.0096	-0.41	12867
Sloughi	Taimyr wolf	-0.0038	0.0100	-0.39	11958
Xinjiang	Taimyr wolf	-0.0031	0.0093	-0.33	12788
Chinese Indigenous Dog 3	Taimyr wolf	-0.0029	0.0093	-0.31	12841
Galgo Español	Taimyr wolf	-0.0028	0.0103	-0.27	10491
Shaanxi 2	Taimyr wolf	-0.0023	0.0097	-0.24	12495
East Siberian Laika	Taimyr wolf	-0.0022	0.0098	-0.23	12532

Table S3. Data figure S8

Table S4

Regions above the 99.5th and 99.95th percentiles of the PBS empirical distribution. Overlapping windows were merged into single regions. Regions below the 0.05th percentile are also shown (see Table S5 for a specific test scanning regions highly differentiated in other dogs relative to sled dogs and wolves).

Table S5

Regions above the 99.95th percentiles of the PBS empirical distribution using other dogs as the focal population. Overlapping windows were merged into single regions. We note AMY2B is not annotated in the chromosome 6 of canFam3.1, but it is immediately upstream of RNPC3 (*19*).

Table S6

Interval enrichment analysis based on the regions in the top 99.95 and 99.5 percentile of the empirical distribution of the PBS, with the sled dogs as the focal population.

References